Heat-shock response in a tropical *Chironomus*: seasonal variation in response and the effect of developmental stage and tissue type on heat shock protein synthesis

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Examination of heat shock induced transcriptional activity in salivary gland polytene nuclei of a tropical *Chironomus*, *C. striatipennis*, revealed nine heat-shock puffs. In 24°C-reared larvae optimal heat-shock response was seen at 39°C, while a 41°C shock was nearly lethal. In a population grown under natural conditions of seasonal variations, the heat-shock response was dependent upon the current ambient temperature. In summer months, response to 39°C was variable, from complete to no induction of heat-shock puffs in different cells. In control glands from larvae growing at 33–36°C in summer, heat-shock genes were not active, although in 24°C-reared larvae, 33°C already caused partial induction. Unlike the 24°C-reared population, a 41°C shock to summer larvae was not lethal. [^{35}S]Methionine-labelled protein synthesis pattern in the summer larvae revealed appreciable accumulation of heat-shock polypeptides in control glands, which possibly autoregulates their further induction and also explains the better thermotolerance of these larvae. In a developmental study of a 24°C-reared population, some heat-shock polypeptides were found to be commonly synthesized at 39°C in all the tissues (salivary glands of larvae; Malpighian tubules of larvae, pupae, and adult; adult ovaries), while other heat-shock polypeptides showed apparent tissue and (or) developmental stage specificity. Heat shock protein 70 was most abundantly synthesized in all the tissues examined.

Key words: temperature shock, thermotolerance, heat-shock polypeptides, polytene chromosomes, puffs.

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L'examen des activités transcriptionnelles induites par des chocs thermiques dans les noyaux de la glande salivaire d'un *Chironomus*, le *C. striatipennis*, a révélé la présence de neuf renflements causés par des chocs thermiques. Chez des larves soumises à une température d'élevage de 24°C, la réponse à une température choc optimale a été vue à 39°C, alors qu'une température de 41°C s'est avérée presque létale. Chez une population croissant dans des conditions naturelles de variations saisonnières, la réponse aux chocs thermiques a varié selon les températures ambiantes. En été, la réponse à une température de 39°C a varié depuis l'absence d'induction à une induction complète de renflements dans différentes cellules. Dans les glandes témoins de larves croissant à 33–36°C en été, les gènes de chocs thermiques n'ont pas été actifs tandis que chez les larves élevées à 24°C, une température de 33°C causait déjà une induction partielle. Contrairement aux populations élevées à 24°C, la température de 41°C ne s'est pas avérée létale pour les larves d'été. Le profil de synthèse de protéines marquées à la méthionine [S^{35}] chez les larves d'été a révélé une accumulation appréciable de polypeptides de chocs thermiques dans les glandes témoins, lesquels assurent possiblement une autorégulation de leur induction ultérieure et expliquent une meilleure thermotolérance de ces larves. Dans une étude de développement de populations de larves à 24°C, certains polypeptides de chocs thermiques ont été communément synthétisés à 39°C dans tous les tissus (glandes salivaires, tubules de Malpighi, pupes et adultes, ovaires d'adultes) alors que d'autres polypeptides ont présenté une spécificité apparente pour certains tissus et (ou) stades de développement. La polypeptide 70 de chocs thermiques a été abondamment synthétisée dans tous les tissus examinés.

Mots clés : chocs thermiques, thermotolérance, polypeptides de chocs thermiques, chromosomes polyténiques, renflements.

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Introduction

The heat-shock response, first documented in *Drosophila* (Ritossa 1962), is now known to be a universal phenomenon and reflects the complexity of the genetic and biochemical adaptations of organisms to cope with a sudden elevation of environmental temperature or certain other stress conditions (Schlesinger 1986; Lindquist 1986).

Earlier studies on the effects of elevated temperature on induction of heat-shock puffs and heat-shock polypeptides in *Chironomus* (Yamamoto 1970; Vincent and Tanguay 1979; Santa-Cruz et al. 1981; Morcillo et al. 1981, 1982; Lezzi 1984; Lezzi et al. 1981, 1984) were confined to European species, adapted to living in a temperate climate. Since the heat-shock condition is relative to the environmental temperature to which the organism is normally adapted, it was of interest to study the pattern of heat-shock response in species living in a tropical climate, where the organism is exposed to a wide range of thermal challenges during summer and winter. The transcriptional and translational changes induced by heat shock in larval salivary gland polytene cells were examined in a tropical species of *Chironomus*. Earlier studies were based on laboratory-adapted populations, maintained at a specific culture temperature. Because temperature is a major environmental variable in poikilothermic organisms, studies were also carried out on a population of *Chironomus* grown under natural conditions of tropical climate to provide insight into any possible environmental influences on the heat-shock response. The present study has revealed certain interesting aspects of seasonal regulation of heat-shock response.

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In addition to examining the heat-shock response in salivary glands of *Chironomus* larvae, the patterns of synthesis of heat-shock proteins at different developmental stages and in different tissues were also studied.

Materials and methods

Cultivation of Chironomus

A laboratory stock of a local species of *Chironomus* (identified by Dr. P. K. Chaudhuri as *C. striatipennis*, personal communication) was used in this study. Originally a large number of chromosomal rearrangements were present in the population. A line was gradually selected that was largely homosequential on both homologs of all the four chromosomes. This line was used for the present study. The larvae were maintained under moderate aeration in plastic dishes containing the culture medium described by Hägele (1975). The larvae were reared at 24 ± 1°C and were fed twice per week with a paste of dried leaves of *Morus alba* and cellulose. The average generation cycle was 6–7 weeks, under these conditions.

For a parallel study with a population grown under natural conditions, a stock derived from the laboratory-reared *C. striatipennis* was maintained in a special insectary where room temperature fluctuated in parallel with the ambient temperature.

Chromosome preparations

Since the chromosome cytology of this species was unknown, mitotic chromosomes were prepared from larval brain ganglia by the air-dry method (Lakhotia and Kumar 1978), while polytene chromosome squashes were prepared from salivary glands of mid fourth instar larvae in the usual manner. Well-spread aceto-orcein stained chromosomes were photographed for the preparation of a reference photomap of polytene chromosomes.

RNA synthesis after heat shock

Temperature shock (TS) to 24°C-reared Chironomus

Salivary glands from 24°C-grown mid fourth instar larvae were subjected to hyperthermic treatments at 33, 35, 37, 39, or 41°C for 60 min, in the inorganic salt constituents of Poels' *Drosophila* culture medium (Lakhotia and Mukherjee 1980). Control salivary glands were incubated at 24°C for 60 min. Subsequently, the treated and control glands were labelled with [^{3}H]uridine (100 μCi/mL (1 Ci = 37 GBq), specific activity 15.2 Ci/mmol; BARC, Bombay) for 10 min at the respective temperatures.

TS to Chironomus *grown under ambient temperature conditions*

As mentioned earlier, a population of *C. striatipennis* was maintained separately under seasonally varying environmental conditions. During a 1-year period, i.e., from January to December 1985, salivary glands from mid fourth instar larvae were excised once or twice every month, heat shocked at 39°C (treated) or incubated at the prevailing temperature for 60 min (control), and labelled with [^{3}H]uridine for 10 min as discussed before. During the peak summer period (June), the glands were also heat shocked at 41°C for 60 min and then labelled with [^{3}H]uridine, as in other cases. The ambient air and culture-medium temperatures were recorded in all these dates.

Autoradiography

After labelling with [^{3}H]uridine, the control and treated glands were briefly fixed in acetic acid – methanol (1:3), stained with 1% aceto-orcein, squashed in 50% acetic acid, and processed for autoradiography with either Ilford L4 or K5 nuclear emulsion in the usual manner. The autoradiograms were exposed for 7–8 days in darkness at 4–6°C, developed with Kodak D-19b developer, and fixed in acid fixer. The slides were then washed, stained with 2% aceto-orcein, and mounted with DPX mountant (BDH Chemicals).

Protein synthesis after heat shock

The protein synthesis patterns under control and heat-shock conditions were studied in different tissues, viz., salivary glands (larva), Malpighian tubules (larva, pupa, and adult), and ovaries (adult).

Labelling of tissue proteins

Salivary glands from 24°C-reared mid fourth instar larvae were heat shocked either at 39 or 41°C, with the control sister salivary glands being incubated at 24°C for 60 min in Poels' salt medium. Salivary glands from larvae reared under environmentally variable conditions were similarly heat shocked in June at 39 or at 41°C. The control sister glands were incubated at the then prevailing ambient temperature (culture water temperature ~36°C) for 60 min. Each sample contained six to eight glands.

For examining the protein synthesis pattern at different developmental stages, Malpighian tubules of larval (mid fourth instar), pupal, and adult *Chironomus* from the 24°C-reared population were used. The choice of Malpighian tubules was based on their continued persistance through metamorphosis (Credland 1980). Four or five pairs of Malpighian tubules were dissected from each stage and were either subjected to heat shock at 39 and 41°C (treated) or were incubated at 24°C (control) in the Poels' salt medium for 60 min.

In addition to Malpighian tubule and salivary gland protein synthesis, protein synthesis was also studied in ovaries. Two or three ovaries from semigravid adult females (36–48 h after emergence) were isolated and heat shocked as described before. Sister control ovaries were incubated at 24°C.

Along with *Chironomus* samples, salivary glands from third instar larvae of *Drosophila melanogaster* were also heat shocked at 37°C for 30 min in Poels' salt medium as described before.

Following the 60-min treatments, all the control and heat-shocked tissues were labelled with [^{35}S]methionine (100 μCi/mL, specific activity >800 Ci/mmol, Amersham Int., U.K.) for 30 min at the respective temperatures. Electrophoresis and fluorography were carried out on different gradients (8–15; 10–15; and 10–18%) of SDS-PAGE slabs as described earlier (Singh and Lakhotia 1988). Equivalent amounts of tissue proteins, as judged by Coomassie brilliant blue staining of gels, were loaded in different wells. Molecular masses were calibrated using protein markers from Sigma.

Results

Chromosomes of C. striatipennis

The diploid mitotic chromosome complement ($2n = 8$) from larval brain ganglia displayed three pairs of metacentric chromosomes (chromosomes I–III) and one pair of small acrocentric chromosomes (chromosome IV). No heterochromatin was apparent in Giemsa-stained preparations of mitotic chromosomes in larval brain ganglia (see inset in Fig. 1).

Salivary gland nuclei of *C. striatipennis* larvae contained four polytene chromosomes: three long elements (chromosomes I, II, and III) and one short one (chromosome IV). Relative lengths of chromosomes I and II were nearly similar, while chromosome III was shorter. No chromocenter was found. The positions of the centromeric regions in polytene chromosomes were not determined. A photomap of polytene chromosomes in salivary gland nuclei from mid fourth instar larvae of *C. striatipennis* is presented in Fig. 1. Since the centromeric positions were not known, the left and right arms could not be distinguished. The subdivisions on the photomap were marked beginning arbitrarily at one end of the chromosome. The flared and lightly stained distal end of chromosome IV (5A/B) was associated with a nucleolus. Two Balbiani rings (BR-1 at 4A, BR-2 at 4B) were found on chromosome IV, depending upon the developmental stage. Generally, BR-1 was the only one seen in mid fourth instar larvae. The nucleolar end of chromosome IV often remained asynapsed.

Heat-shock puffs

On the basis of morphological features and [^{3}H]uridine uptake patterns (Figs. 2 and 3), nine heat-shock puffs were identified in *C. striatipennis*. Chromosome I had two heat-

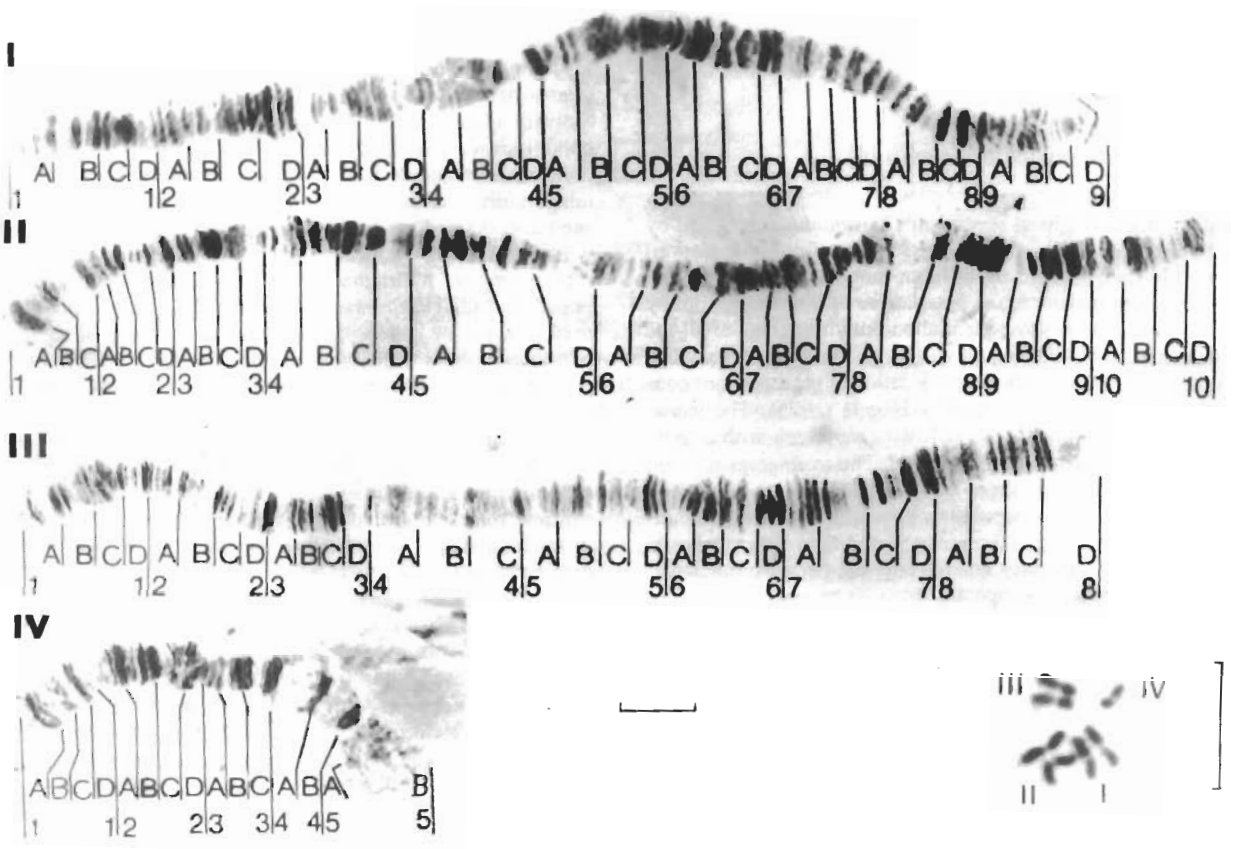


FIG. 1. Photomaps of polytene chromosomes from salivary glands of mid fourth instar larvae of *Chironomus striatipennis*. Inset in the lower right corner shows a diploid metaphase from larval brain ganglia. The scale bars represent 10 μm.

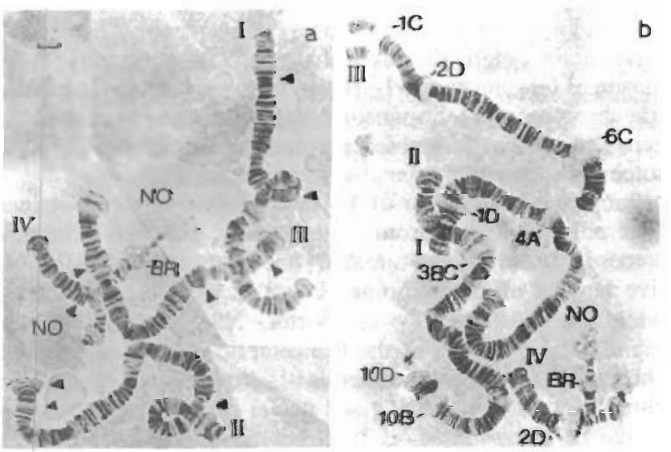


FIG. 2. Photomicrographs of aceto-orcein stained polytene nuclei showing the heat-shock loci in (*a*) control and (*b*) heat-shocked (39°C) glands. Map positions of the induced puffs are indicated in Fig. 2*b*, while the corresponding sites in the control are indicated by arrowheads in Fig. 2*a*. BR, Balbiani ring on chromosome IV; NO, nucleolus. Scale bar represents 10 μm.

shock puffs, 1D and 4A; 4A was transcriptionally more active. Chromosome II contained three heat-shock puffs, 3BC, 10B, and 10D, with 3BC being the major one and the 10D puff having a telomeric location. Chromosome III contained the largest heat-shock puff at the 6C region, along with two others at 1C and 2D. Morphologically, the III-6C locus in heat-shocked glands resembled a Balbiani ring. Chromosome IV contained only one major heat-shock puff, at the 2D locus. The developmentally active BRs generally collapsed in heat-shocked glands. Occasionally some other sites also developed morphological puffs after heat shock (e.g., 9A on chromosome I), but since they did not show any significant [^{3}H]uridine incorporation, such sites were not considered as heat-shock loci.

[^{3}H]Uridine incorporation after heat shock at different temperatures in 24°C-reared Chironomus

The effect of heat shock at different temperatures (33–41°C) on transcription in polytene nuclei was studied. The numbers of silver grains on each of the heat-shock puffs, and a segment of chromosome III (from 7A to 8D) that lacked any heat-shock puff, were counted. The data on [^{3}H]uridine incorporation at these sites after different temperature treatments (33–39°C) are presented in Fig. 4. The general chromosomal transcription (as evidenced by grain count data for the chromosome III segment) was affected minimally at 33 and maximally at 39°C. Heat shock for 60 min at 37°C caused only a partial inhibition of general transcription. The normal developmental puffs were still active at 37°C but were nearly completely regressed at 39°C. The rate of nucleolar transcription was not significantly affected (data not presented), but the distribution of silver grains over the nucleolar area was affected since after heat shock the [^{3}H]uridine uptake in the nucleolus was often restricted to its proximal part (see Fig. 3).

The different heat-shock puffs responded differently to hyperthermic treatments. The heat-shock puffs on chromo-

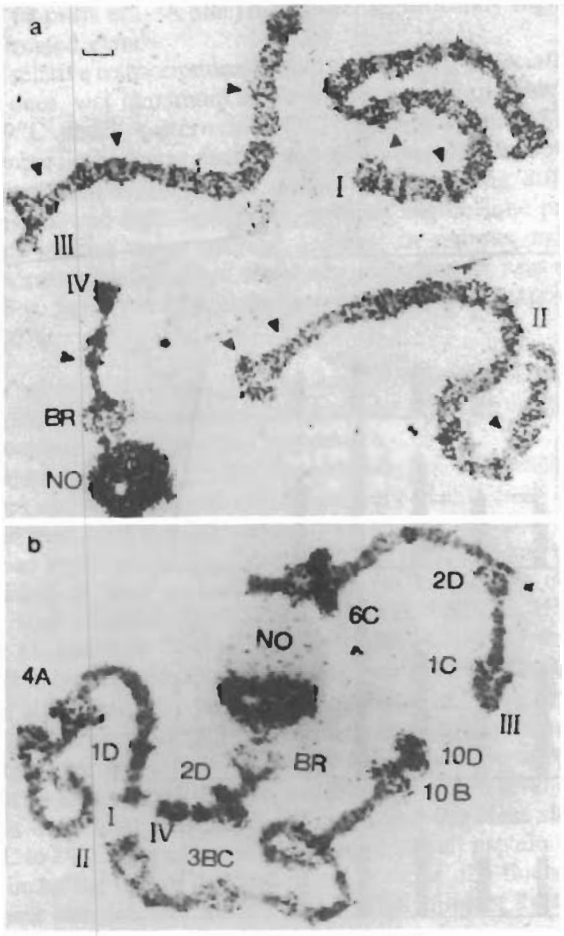


FIG. 3. [^{3}H]uridine-labelled polytene chromosomes from (*a*) control and (*b*) heat-shocked (39°C) glands. Other details are as in Fig. 2. Scale bar represents 10 μm.

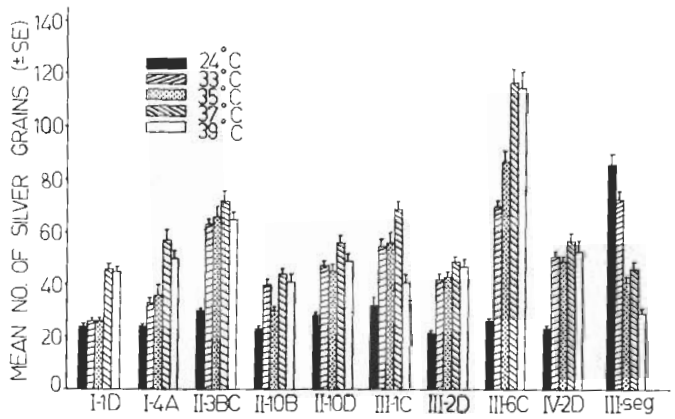


FIG. 4. Histogram showing [^{3}H]uridine incorporation (mean number of silver grains ± standard error, ordinate) on different heat-shock loci and the 7A−8D segment of chromosome III (abscissa) at different temperatures. For each data point, 15−22 nuclei were scored.

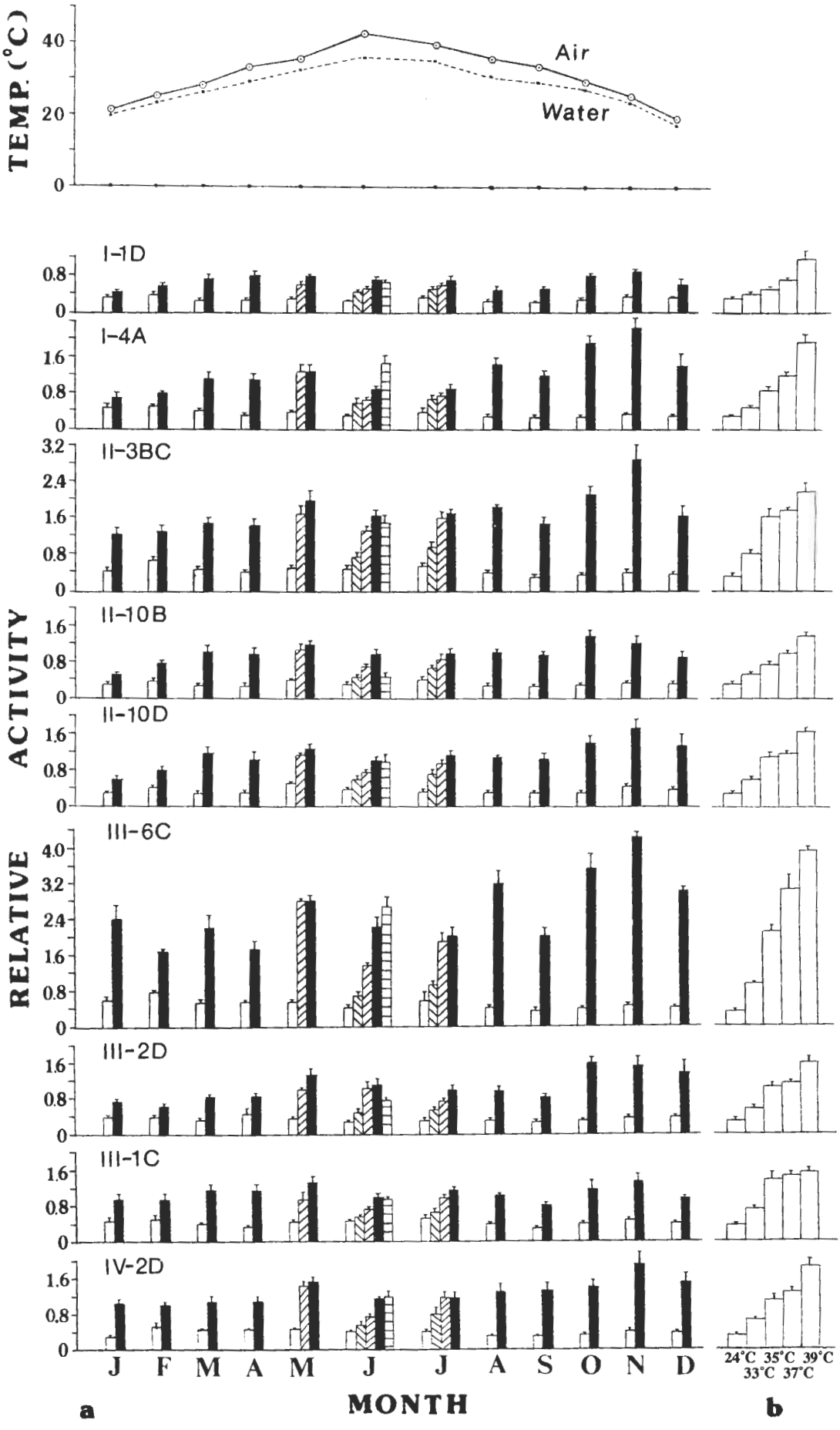
some I (1D and 4A) were not or much less affected at 33 and 35°C, while those on other chromosomes were significantly induced, even at 33°C. Unlike the other heat-shock loci, the BR induced at III-6C showed a remarkable increase in its activity with increasing temperature treatment (Figs. 4 and 5*b*). The mean numbers of grains on different heat-shock puffs at 39 and at 37°C were not significantly different (see Fig. 4). However, since the chromosomal transcription was affected maximally at 39°C (Figs. 4 and 5*b*) while the heat-shock puffs were nearly equally active at 37 and 39°C, the relative activity (mean ratio of grain counts on a TS puffs and the reference chromosome segment of the same nucleus) of each of the TS puffs was higher at 39°C (see Fig. 5*b*). In view of these, it is considered that 39°C elicits an optimal TS response in this species.

A 60-min heat shock at 41°C to salivary glands from 24°C-reared larvae resulted in severe cellular damage, with most nuclei appearing highly pycnotic and condensed. No [^{3}H]uridine incorporation was detectable in these glands.

[^{3}H]uridine incorporation after heat shock to Chironomus *grown under ambient conditions*

Studies on the induction of heat-shock puffs in a population of *Chironomus* grown under natural conditions of seasonally varying temperature revealed some interesting aspects of the heat-shock response. Figure 5*a* shows the relative activity of different TS puffs in different months and the recorded temperature of the air and the water medium in which the larvae were growing at the time of heat-shock tretment in each month of the year. Comparison of the relative transcriptional activities of different TS loci (see Fig. 5) revealed that the TS response in this population varied in relation to ambient temperature. In summer months (June and July), when the larval culture medium temperature was already between 34 and 36°C, the induction of the TS loci in response to 39°C treatment showed three distinct patterns (see Figs. 5 and 6). In some nuclei (type I (T-I)), induction was complete with all the TS puffs transcriptionally active (Figs. 5 and 6); in some other nuclei (type II (T-II)), minor loci (viz., III-1C, III-2D, II-10D, II-10B, I-1D) were not induced but the major ones (viz., IV-2D, III-6C, II-3BC, I-4A) were more or less induced; in the rest of the nuclei (type III (T-III)), none of the TS loci were induced, as indicated morphologically or by [^{3}H]uridine labelling. The T-I, T-II, and T-III nuclei could not apparently be correlated with different regions of gland or to different size classes of nuclei.

In May, when the ambient temperature was less than in June and July, the T-III nuclei were not seen and the difference between T-II and T-I was less marked, particularly for the major TS puffs. In June, when the ambient temperature was maximum, the relative activity of nearly all TS loci was less in T-I as well as T-II nuclei (Fig. 5*a*). Comparison of the mean grain counts on different TS loci and the reference chromosome segment in preparations made in May, June, and July (Fig. 7) also confirmed the distinction between T-I, T-II, and T-III induction. Such variability in TS response was not found in other months of the year. In the 24°C-reared laboratory-adapted population also, the T-II or T-III patterns of induction after heat shock at 33, 35, 37, or 39°C were never seen. Interestingly, in June, salivary gland cells survived at 41°C heat shock and responded with a more or less typical TS response (T-41, in Figs. 5, 6, and 7). General chromosomal transcription was drastically inhibited (~70%) at 41°C; the mean grain counts on all TS loci after 41°C TS were also significantly less than in 39°C-shocked glands (see Fig. 7). However, when relative activities were compared (Fig. 5*a*), the values for 41°C-treated glands were nearly equal to those in 39°C-shocked glands; in fact the relative activities of the


TEMP. (°C)
40
20
0
Air
Water
ACTIVITY
RELATIVE
I-1D
I-4A
II-3BC
II-10B
II-10D
III-6C
III-2D
III-1C
IV-2D
J F M A M J J A S O N D
MONTH
24°C 35°C 39°C
33°C 37°C
a
b

major TS puffs at I-4A and III-6C were significantly higher in 41°C-treated glands.

The relative transcriptional activity of TS loci, especially the major ones, was maximum in November and comparable with the 39°C shock pattern in 24°C-reared population. After November, it began to decline and fluctuate with the ambient temperature. The TS loci in control glands during different months showed little variability, without any definite pattern in their relative transcriptional activity. In summer months, the control glands did not show any induction of heat-shock loci (Fig. 5*a*), even though the larvae were already exposed to 34–36°C.

Effect of heat shock on protein synthesis in larval salivary glands in 24°C-reared and ambient temperature reared summer population of Chironomus

Comparison of the [^{35}S]methionine-labelled polypeptides in control and heat-shocked (39°C) salivary glands from 24°C-reared mid fourth instar larvae revealed that the heat shock induced synthesis of about eight or nine polypeptides, named according to their molecular masses (in kilodaltons (kDa)), viz., HSP 82, HSP 72, HSP 70, HSP 60, HSP 36, HSP 28, HSP 24, and HSP 18 (Fig. 8). Occasionally HSP 27 was also found to be induced after heat shock (see Fig. 9). The synthesis of HSP 70 was by far the most abundant at 39°C. The normal translational activity in *Chironomus* was only marginally inhibited by heat shock at 39°C. A polypeptide species of about 33 kDa was particularly notable for its high level of synthesis even after heat shock (Figs. 8 and 9). Heat shock at 41°C to 24°C-reared larval glands inhibited all protein synthesis. including that of the heat-shock proteins: the fluorograms of these samples showed only traces of labelling of the 82- and 70-kDa HSPs (Figs. 8 and 9).

Protein synthesis in control and heat-shocked (39 or 41°C) salivary glands from 24°C-reared and ambient temperature reared summer-month larvae revealed significant differences (Fig. 8). An interesting aspect of heat-shock response in summer larvae was that the control glands were already synthesizing HSPs at detectable levels (compare lane D with A, Fig. 8). Heat shock at 39°C to salivary glands from summer larvae further stimulated the synthesis of the HSPs, especially the higher molecular mass species. Ongoing normal protein synthesis was not inhibited at 39°C. Another interesting observation was that while 41°C shock drastically inhibited protein synthesis in the 24°C-reared population, the summer larvae continued to show the normal heat shock protein synthesis pattern even at 41°C. The normal ongoing translational activity was also not significantly affected by the 41°C treatment in these larvae. Apparently the pattern of protein synthesis in summer larvae at 41°C resembled the 39°C shock pattern of 24°C-reared larvae (Fig. 8).

Effect of heat shock on protein synthesis in different tissues of different developmental stages

Studies on the effect of heat shock on HSP synthesis in larval, pupal, and adult stages revealed that some HSPs were

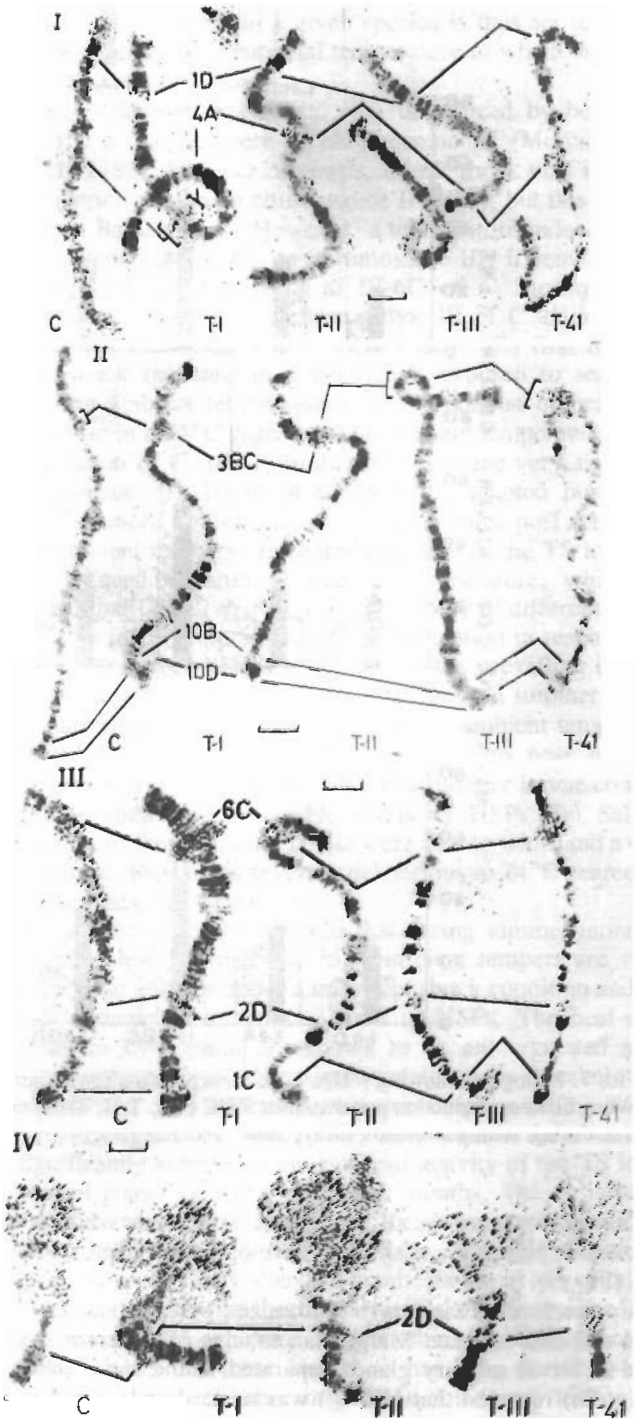


FIG. 6. Photomicrographs of [^{3}H]uridine-labelled polytene chromosomes showing different patterns of heat-shock puff induction in ambient temperature reared summer larvae in chromosome I, chromosome II, chormosome III, and chromosome IV. T-I, T-II, and T-III correspond to the types described in text. T-41 corresponds to chromosomes from glands heat shocked at 41°C. The scale bars represent 10 μm.

FIG. 5. Histograms showing relative transcriptional activity (means of ratio of grain counts over a puff site to that over the reference chromosome segment) of different TS puff sites in control and heat-shocked salivary glands of larvae from (*a*) ambient temperature reared and 24°C-reared population of *C. striatipennis*. (*a*) The relative activities of each TS puff in different months (abscissa, January–December) in control (▯) glands (at the prevailing ambient temperature shown in upper part of the figure) and after heat shock at 39°C (▮). Because the TS response in individual nuclei during summer months (May, June, and July) varied, the mean relative activities in T-I (▮), T-II (▒), and T-III (▒) nuclei (see text) in samples for these months are shown separately. Relative activities of TS puffs after a 41°C shock (▒) in June are also shown. (*b*) Relative activities of different TS puff sites at 24°C (control) and after heat shock at different temperatures (as indicated on abscissa) to glands from 24°C-reared larvae.

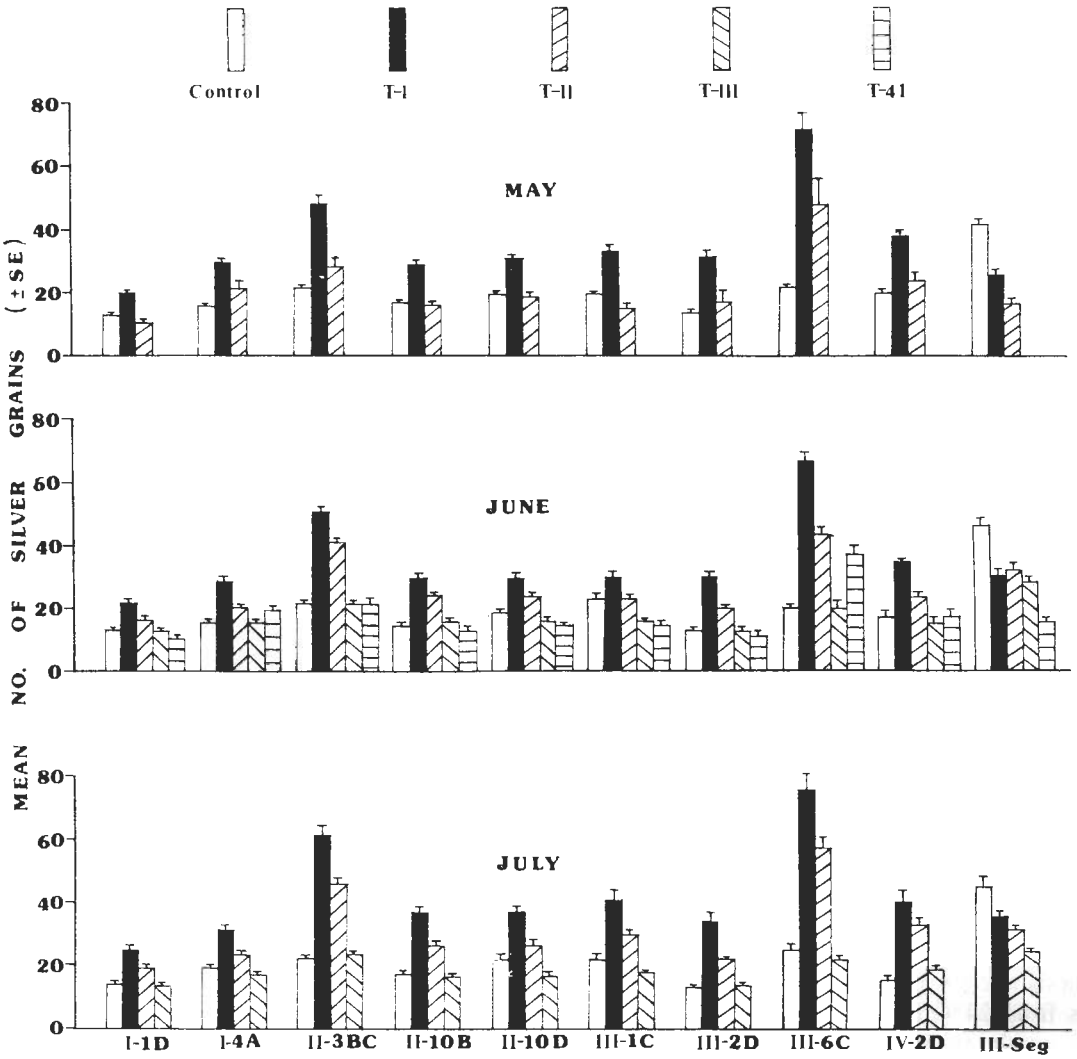


FIG. 7. Histogram showing [^{3}H]uridine incorporation (mean number of silver grains ± standard error, ordinate) on heat-shock loci in nuclei showing different induction patterns after 39°C (T-I, T-II, T-III) or 41°C (T-41) heat shock to salivary glands from ambient temperature reared larvae during summer months (May, June, and July).

commonly induced in all the tissues examined, while some showed apparent tissue and (or) developmental stage specificity (Fig. 9).

Comparison of the newly synthesized polypeptides in control and heat-shocked Malpighian tubules of different stages and in larval salivary glands separated in the same slab gel (Fig. 9*a*) revealed that HSP 70 was most abundantly synthesized in all the tissues (Fig. 9). Other common HSPs seen in all the tissues examined were the 82-, 72-, 36-, 28-, and 24-kDa species (Fig. 9; Table 1).

Malpighian tubules from different developmental stages showed some stage-specific HSPs, viz., 66 kDa in adult and 60, 55, and 50 kDa in pupa. In addition, pupal and larval Malpighian tubules synthesized two common HSPs, namely, HSP 30 and HSP 23, which wre not seen in the adult; on the other hand a 29-kDa HSP was seen only in adult and pupal Malpighian tubules (Fig. 9; Table 1). Although the larval Malpighian tubules and salivary gland protein samples were from the same individuals, there were some notable differences in HSP induction between these two tissues: HSP 60 and HSP 18 were seen in the salivary gland samples but not in larval Malpighian tubules. Interestingly, however, a HSP 60 was seen in pupal Malpighian tubules (Fig. 9*a*).

Heat shock at 41°C drastically inhibited protein synthesis in all tissues except the pupal Malpighian tubules. Some pupal stage specific HSPs, viz., HSP 60, HSP 55, and HSP 50, were in fact often found only in the 41°C-treated samples, although occasionally these were seen in 39°C-treated samples as well (not shown). In other tissue samples, only the HSP 82 and HSP 70 were detectable as very faint bands in the fluorograms after a 41°C treatment, although the corresponding lanes in the Coomassie brilliant blue stained gel (not shown) showed comparable levels and patterns of polypeptides.

Examination of protein synthesis in adult ovaries (Fig. 9*b*) revealed that a 39°C heat shock induced synthesis of most of the HSPs commonly found in other tissues without a significant inhibition of the synthesis of normal proteins. However, most of the HSPs, such as 82, 72, 70, 28, 27, etc., were also synthesized at detectable levels in control ovaries. Heat shock at 39°C subsequently increased the synthesis of these HSPs, while the other high and low molecular weight HSPs, viz., 66, 36, 24, and 18, were newly induced. No HSP appeared to be unique to ovaries as evident from a comparison of different tissues examined in this study (see Table 1).

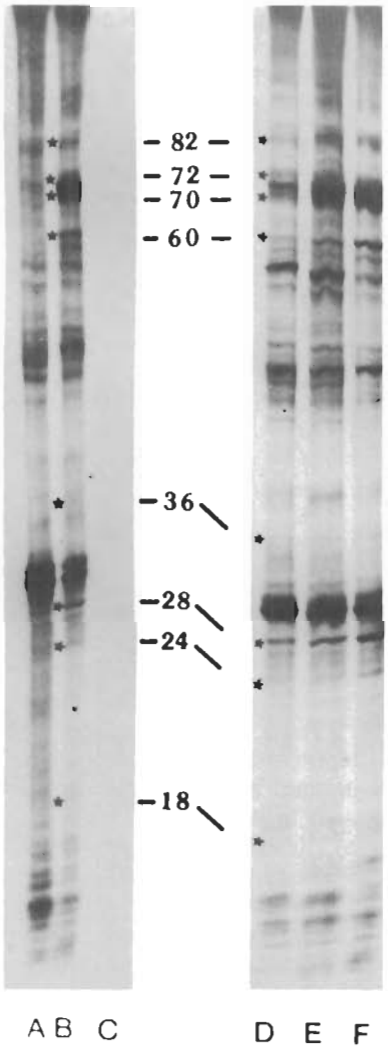


FIG. 8. Fluorogram of [^{35}S]methionine-labelled proteins in control (A, D) and heat-shocked (39°C: B, E; 41°C: C, F) salivary glands from 24°C-reared (A, B, C) or ambient temperature (~36°C) reared (D, E, F) larvae of *C. striatipennis* during June.

Except in 24°C-reared larval salivary glands, a polypeptide comigrating with HSP 70 could be detected at low levels even under non heat shock conditions in all other tissues examined (Fig. 9). In control larval Malpighian tubules, a significantly higher level of synthesis of a 70-kDa polypeptide was noticed.

Discussion

As expected from previous studies on other species of *Chironomus* and *Drosophila*, the polytene nuclei of *C. striatipennis* responded to elevated environmental temperature in a characteristic manner, viz., inhibition of general chromosomal transcription and activation of a specific set of heat-shock loci. However, the present results show that unlike in the European chironomids (Lezzi 1984; Lezzi et al. 1981, 1984; Morcillo et al. 1981, 1982; Santa Cruz et al. 1981), heat shock at 37°C was not as effective in inhibiting general transcription as at 39°C. The heat-shock puffs themselves, however, were equally induced at 39 and 37°C. The persistence of chromosomal transcriptional activity at significant levels at 37°C in *C. striatipennis* polytene cells appears to reflect adaptation of this species to a warmer climate. Threshold level for the heat-shock response in a given species is thus set in relation to the general environmental temperature to which the organism is exposed in nature.

In *C. thummi*, a Balbiani ring is induced by heat shock (T-BR) at the telomere of chromosome III (Morcillo et al. 1981, 1982). In *C. striatipennis*, a heat-shock puff is seen at telomeric location on chromosome II (10D), but this does not form a Balbiani ring. However, a temperature-induced Balbiani ring is seen at 6C on chromosome III. It remains to be shown whether the T-BR at III-6C of *C. striatipennis* is homologous to T-BR of chromosome III of *C. thummi*.

To our knowledge the present study is the first report of heat-shock response in a population exposed to seasonally varying ambient temperatures. A comparison of heat-shock response in a 24°C-reared and an ambient temperature reared population of *C. striatipennis* revealed some very significant differences. (*i*) While in a laboratory-adapted population, 33°C induced a moderate level of heat shock puff activity, in the ambient temperature reared population, the TS loci were not induced by ambient summer temperature, which was higher than 33°C. (*ii*) The relative activity of different TS loci in the ambient temperature reared population in response to a 39°C heat shock varied in relation to the prevailing environmental temperature. (*iii*) The TS response in summer months in individual cells of salivary glands of ambient temperature reared larvae varied, with no induction to near complete induction in different nuclei. (*iv*) The summer larvae constitutively synthesized detectable levels of HSPs. (*v*) Salivary gland cells from summer larvae were able to withstand a 41°C exposure, which was severely deleterious to 24°C-reared laboratory-adapted larvae.

It appears from these results that during summer months, a more or less continuously high ambient temperature would keep these animals under a mild heat shock condition and thus they accumulate an optimal level of HSPs. The heat-shock genes in *Drosophila* are known to be autoregulated at the transcriptional level in that accumulation of a threshold level of HSPs in cells inhibits further transcription of the TS loci (DiDomenico et al. 1982). This may explain the absence of significantly elevated transcriptional activity of the TS loci in control glands during the summer months. The reduced and variable induction of heat-shock loci in response to 39°C in May, June, and July (the T-I, T-II, and T-III response in different nuclei) may also be a manifestation of autoregulation of these loci. The fact that the salivary glands from June larvae survive a 41°C exposure, which otherwise is more or less lethal, shows that these larvae, as has been shown to occur in *Drosophila* and other organisms (Lindquist 1986), have acquired thermotolerance as a result of continuous exposure to high ambient temperature during these months. The constitutive synthesis of HSPs in summer larvae thus appears to be a case of homeostasis.

The pattern of HSP synthesis in salivary glands of 24°C-reared larvae follows the usual pattern seen in *Drosophila* and other species of *Chironomus* (Lindquist 1986; Carretero et al. 1986; Vincent and Tanguay 1979), although some differences in molecular masses of the HSPs from those recorded in other species of *Chironomus* were apparent. However, such minor differences in the molecular masses are known from earlier studies in the European species of *Chironomus* as well as in other organisms. Coelectrophoresis of labelled proteins in salivary glands of *D. melanogaster* and *C. striatipennis* in the same slab revealed molecular mass identity of the major HSPs,

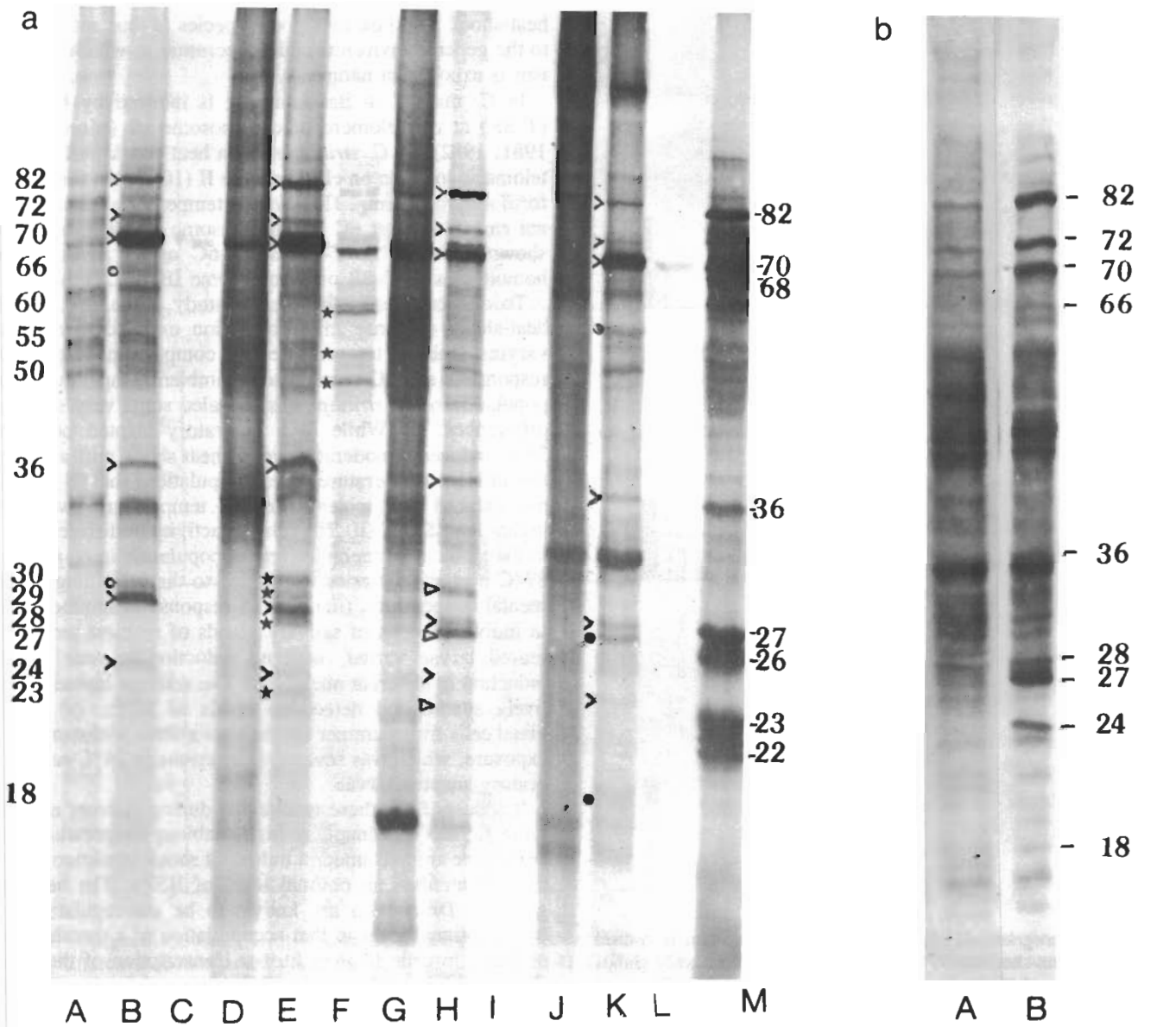


FIG. 9. (*a*) [^{35}S]Methionine-labelled protein synthesis patterns in Malpighian tubules (adult: A, B, C; pupa: D, E, F; larva: G, H, I) and larval salivary glands (J, K, L) of *C. striatipennis* following heat shock at 39°C (lanes, B, E, H, and K) or at 41°C (lanes C, F, I, and L) along with corresponding controls (24°C) in lanes A, D, G, and J. Lane M shows heat shock (37°C) protein synthesis pattern in larval salivary glands of *Drosophila melanogaster*. Tissue and stage specificity of the HSPs is indicated: >, common HSP;s; ○, adult-specific HSPs; ★, pupa-specific HSPs; ⩾, larval Malpighian tubule specific HSPs; ●, larval salivary gland specific HSPs. (*b*) Patterns of protein synthesis in ovaries of adult *Chironomus* at 24°C (lane A) and after 39°C (lane B).

such as 82, 70, and 36 kDa. Also as expected from other studies (Lindquist 1986), the HSP 70 species was the most abundant in *C. striatipennis* in all the tissues examined.

A significant feature of the present study was the comparison of the pattern of HSPs in different tissues at different stages of development. There have been a few studies on this aspect in *Drosophila* and some other systems (Lindquist 1986; Schlesinger 1986; Bond and Schlesinger 1987) but none in *Chironomus*. The present results revealed that while some of the HSPs (e.g., 82, 72, 70, 36, 28, and 24) were ubiquitously induced in all the cell types tested, others were induced only in certain tissues and (or) at specific developmental stages. At present it is not known if all these different HSPs (15 altogether, see Table 1) are products of different genes or result from differential processing of the primary transcripts and (or) modification of the translational product. These stage-specific HSPs may belong to multigene families (HSP cognates, Craig et al. 1982) which are regulated at the transcriptional and (or) translational levels. The synthesis of particular HSPs at specific stages may also be due to modulation by hormonal factors. In *Drosophila*, expression of the low molecular mass HSP genes is under the dual control of heat shock and ecdysterone, resulting in a constitutive expression at the pupal and other stages (Bond and Schlesinger 1987). The somewhat tissue specific pattern of constitutive synthesis of several HSPs in non heat shocked cells of the 24°C-reared population is in agreement with similar findings reported in *Drosophila* and other organisms (Zimmermann et al. 1983; Cheney and Shearn 1983; Lindquist 1986; Bond and Schlesinger 1987).

Unlike many other organisms (Lindquist 1986), *Chirono-*

TABLE 1. Stage- and tissue-specific patterns of synthesis of HSPs

HSP (kDa)	Adult		Pupa,	Larva	
	OV	MT	MT	MT	SG
82	+	+	+	+	+
72	+	+	+	+	+
70	+	+	+	+	+
66	+	+	−	−	−
60	−	−	+	−	+
55	−	−	+	−	−
50	−	−	+	−	−
36	+	+	+	+	+
30	−	−	+	+	−
29	−	+	+	−	−
28	+	+	+	+	+
27	+	−	+	+	+
24	+	+	+	+	+
23	−	−	+	+	−
18	+	−	−	−	+

NOTE: + and −, presence or absence of a particular HSP; OV, ovary; MT, Malpighian tubule; SG, salivary gland.

mus did not show a significant inhibition of normal translation after a heat shock at 39°C. Lack of translational inhibition perhaps reflects a tropical adaptation of these insects. Heat shock at 41°C, on the other hand, drastically reduced the overall protein synthesis in all the tissues except in pupae, where inhibition was relatively less compared with larval and adult stages. In *Chironomus*, the pupal stage seems to be thermally better adapted. There are other instances of hardiness of the pupal stage. *Chironomus* pupae are well known for their capacity to withstand extreme environmental conditions and to emerge only when favourable conditions are restored. In the natural habitats, pupae of this tropical species of *Chironomus* were noted to withstand different kinds of stresses, such as desiccation, etc. This may be a possible biological explanation for the pupae showing relatively more tolerance to 41°C shock in *C. striatipennis*. In *Drosophila*, hormonally induced accumulation of small HSPs at pupal stages is known to confer stage-specific tolerance to higher temperature (see review by Bond and Schlesinger 1987). In *Chironomus*, maximum numbers of small HSPs were also induced in pupal Malpighian tubules and these too may be involved in conferring a greater thermotolerance to the pupal stage.

The results of the present study on different aspects of heat-shock response show that a tropical species of *Chironomus* responds optimally at 39°C, instead of 37°C, as in the European *Chironomus* species adapted to a colder climate. Moreover, the homeostatic mechanism of the tropical species also allows normal development of *Chironomus* at a continuously high ambient temperature (33−35°C). The heat-shock response seems to have an important role in this homeostasis. Thus, the heat-shock response not only serves as a short-term protection mechanism against a sudden elevation of environmental temperature but also has a significant long-term role in permitting the organism to adapt to the severe summer temperatures of the tropics.

The heat-shock response seen in ambient temperature grown larvae during the other months of the year when the environmental temperature was lower was very similar to that seen in the 24°C-reared larvae but different from the response in the summer months. Since the same population was maintained throughout the year at the prevailing ambient temperature, the different TS response in summer and other months shows that the expression of heaet-shock genes in this tropical insect has not become constitutive but has remained inducible.

This study also suggests that the expression of TS genes is not necessarily always coordinated, but the induction can be tissue and developmental stage specific (also see Bond and Schlesinger 1987). It is difficult to make any definite correlation between the specific expression of certain genes at different stages of development, becaue studies to date have not been able to elucidate fully a specific role for HSPs so far as differentiation and development are considered (Bond and Schlesinger 1987; Pelham 1988). *Chironomus*, with its different species adapted to very diverse climates, provides a good tool for such studies.

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